Ph.D SYNOPSIS

Title: "Evaluation of selected natural substances for their anti- breast cancer activities".

1.0 Introduction:

Cancer is a world's second leading cause of the death and numbers of the cancer affected cases are continuously increasing. In this year (2016) alone in USA the estimated new cases of the cancer are about 16,85,210 and morbidity cases are of 5,95,690 cases (Cancer Facts & Figures 2016). In India, for the year 2016 estimation of morbidity due to the cancer is about 12,19,649 (Murthy et al.,2008) and one of the reason for this increased number is the percentage of cancer detection and survival after the treatment is very less than the Europeans and Americans. Cancer is a molecularly heterogeneous disease and it can be induced in the any part of the body. Cancer is initiated by the genetic mutations in the cells which causes failure of the 'cell cycle' control. These abnormal cells (mutated) become immortal and generate their progeny continuously, in some cases they form a tumor and in some cases they grow without the tumor.

1.2 'Breast cancer' mostly detected cancer in women:

In the United States, breast cancer is the second leading cause of death in women and for the year 2016, breast cancers new cases are estimated about 2,46,660 and death risk 'number' is estimated about 40,450. In the year 2012 in India breast cancer detected cases were 1,44,937 and morbidity were 70,218. It indicates that, in India from every two women who had diagnosed breast cancer from them one woman died. This morbidity number in India is very high than the other countries like USA and China where more than 70-80% cases survive after the diagnosis of breast cancer and in India more than 50% cases pass away. These numbers of survival rate are very important for designing a plan for cure, awareness and proper guidance for preventing its prevalence.

1.3 Types of breast cancers:

Breast cancers can be classified by stage, pathology, grade, and expression of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor (Her2/neu) (Holliday et al., 2011) and regarding to these characteristics breast cancer is overall divided as Luminal A tumors (30-70%), Luminal B tumors (10-20%), Triple negative/basal-like (15-20%) and HER2 (5-15%). In these types of breast cancers hormonal receptors positive cancer can be treated by the chemotherapy or hormonal therapy but for the triple negative cancers only

chemotherapy can be useful because of these types of cells do not have any receptors on the cell membranes.

1.4 Current breast cancer treatment approaches and limitations:

Physical methods include surgery, radiation and chemotherapy. Surgery is preferred many times for the removal of tumor. Chemotherapy is a type of treatment in which certain type of toxic and chemically synthesized drugs used for the inhibition of cancer cell growth. Use of the chemotherapy for the cancer was started in the year 1950s and 'combination chemotherapy' was started in the year 1970. Shockingly, due to the current therapy which is not a 'curative' and it is only 'cell killing' therapy more than half a century later there is no more changes in patient survival seen (Cardoso et al.,2002).

Generally in chemotherapy natural drugs like anthracyclines (e.g. doxorubicin) and the taxanes (e.g. paclitaxel) has been regularly used for treating the non hormonal cancers, and for hormonal cancer therapy (ER, PR and HER2 positive) treated with the drug trastuzumab alone or with these taxanes. Presently prescribed chemotherapeutic and chemopreventive drugs which are showing anti-proliferative effects via an arrest of 'cell cycle' and which is a important mechanism for inducing normal cell death. So, "cell cycle-mediated apoptosis" is a increasing attention for making the novel agents in cancer therapy and regarding to this certain natural agents, are believed to function via this pathway (Ahmad et al., 2000). Also there is a biological therapy in which living organism, substances derived from living organisms (Monoclonal antibodies, cytokines, therapeutic vaccines, the bacterium bacillus Calmette-Guérin, cancer-killing viruses, gene therapy, and adoptive T-cell transfer) and synthetic versions of such natural substances are used to treat cancer.

2.0 Review of literature:

Nature is an ancient pharmacy, which is used by utmost people of the many countries and due to this many countries prefer natural substances for treating various diseases and similarly 'Ayurveda' is the ancient medicinal therapy which is used for treating various human diseases & it has been practiced in India for over 5000 years back. In India as per ICMR guidelines of the Drug and Cosmetics rules state that Ayurvedic formulations which are prepared by the traditional methods they do not have to undergo tests for safety and efficacy if, they are used for diseases specified in the original texts (ICMR, 2006) and also in the United States, Ayurvedic products are regulated as a dietary supplements. In 'Ayurveda' there are two main systems are

well known called Charaka Samhita and Sushruta Samhita, there are 341 plants are reported in Charaka Samhita and in Sushruta Samhita 385 plants are reported for treating various human diseases, many of them are not tested for the anti breast cancer activity.

'Natural substances' generally regarded as being synonymous with 'secondary metabolite', and these organic substances are relatively of small molecular weight (<3,000 Daltons). These substances can be secreted from animals, microorganisms, marine life forms and terrestrial plants. Species of the tropical flowering plants estimated diversity is of 2, 00,000 to 4, 50,000 in our biosphere (Prance et al., 2000 and Govaerts 2002) and from them only about 5,000 plant species (1.5-2%) have been in detail studied for the medical benefits (Wink et al., 2007), also it has been calculated that as many as 60,000 species of flowering plants will have become extinct before the year 2050.

Primary metabolites of plants (sugars, fats, amino acids, etc.) serve as primarily source of the nutrition and as starting materials for the further biosynthesis and secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids and essential oils are important for the protection and thus they are used as 'weapons' in the fight against pathogens such as viruses, bacteria, and protozoan's. Although these produced metabolites not useful directly to the plant for their diet but these metabolites have essential evolutionary importance for the biological "fitness" of the plants. Main importance of the secondary metabolites for the humans is due to their pharmacological properties and these properties are considered as an evolutionary byproduct of the nature (Fulda and Efferth., 2014). Today there are numerous reasons of using phytochemicals in the medicines but mostly important is 'ability to induce apoptosis' in cancer cells without causing cytotoxic effects on other body parts.

Cancer damage can be prevented or limited by dietary antioxidants which are present abundant in plant phytochemicals and by using this natures gift cancer can be prevented (Simopoulos 2001). These dietary/edible phytochemicals which reduces cancer risk, some of them are Curcumin, Capsaicin, Gingerol, Epigallocatechin-3-gallate, Genistein, Sulphoraphane, Indole-3-carbinol, Diallylsulphide, Caffeic acid phenethyl ester and Resveratrol. Also there are various natural molecules of plant origin which are approved by FDA as anti-cancer drugs and they are in the market ex. Vinblastine, Paclitaxel (Taxol) etc.

Marine floras, such as bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes are extremely important oceanic resources, constituting over

90% of the oceanic biomass (Simmons et al., 2005).They are taxonomically diverse, largely productive, biologically active, and chemically unique offering a great scope for discovery of new anti-cancer drugs. It has been estimated that 1.5 million species of fungi exist in the world and of these; only 70,000 have been subjected to taxonomic studies (Kinghorn et al. 2009). Hence, the vast majority of fungi have never been tested for any kind of biological activity could become more useful for cure the cancer.

3.0 Objectives of research/ proposed hypothesis:

1. Collection, identification, authentication, quality control of the selected medicinal plants and Ayurvedic preparations.
2. *In vitro* and *in vivo* assessment of the selected medicinal plants and different Ayurvedic preparations for their anti breast cancer activities.
3. Development of novel, safe, effective and evidence based anti-breast cancer natural formulation complementary for improving the efficacy of current therapeutic regimes.

4.0 Methodology to be adopted:

1. Selection and collection of the medicinal plants and Ayurvedic preparations from Ayurvedic literatures, interviewing local healers, searching online Ayurvedic data bases etc.
2. Preliminary screening of the selected medicinal plants and Ayurvedic preparations for their anti-proliferative activities against the human breast cancer cell lines.
3. Evaluation of anti-breast activity of selected promising samples (individual and in synergy) by performing key experiments like cell proliferation assay (MTT assay), detection of anti-angiogenic properties (CAM assay), apoptosis (Annexin V assay), cell cycle assay (PI staining) and 3D cell culture assay.
4. Demonstration of *in vivo* anti-breast cancer activity by using the Human Xenograft Tumor Models.

5.0 Importance of study/Society application:

The main disadvantage of the synthetic drug is these types of drugs are not target specific and it causes side effects to the cancer subject. However natural therapies (Ayurvedic) which use plants or plant derived natural products are being beneficial to combat many human diseases along with cancer. The search for anti-cancer agents from plant sources (natural) started in the 1950s when discovery and development of the *Vinca* alkaloids (vinblastin and vincristine), and the isolation

of the cytotoxic podophyllotoxins was carried out. After that the National Cancer Institute (NCI) of the United States of America (USA) had screened about 1,14,000 extracts from an estimated 35,000 plant samples against the various cancers. Due to this research from the year 1983 and 1994 total 92 anticancer drugs are commercially available for the cancer therapy and from them approximately 62% are of the natural origin.

This present study is also designed for the finding new natural origin substances which possess ability of inducing anti proliferative activities in the cancer cells along with low degree of cytotoxicity towards the normal cells and specifically in the tumor cells of Indian subjects.

In this study we will use current and advanced *in vitro* and *in vivo* techniques e.g. MTT assay, Cell death analysis by Flow Cytometry, 3D cell culture, other cell death related experiments and mouse models. Hence, this work is conducted on the aspect with a view to provide a baseline information for promoting the undiscovered natural resources of flora and fauna (plants, marine flora and the micro-organisms) and Indian Ayurvedic therapeutics which could become safer, effective, novel anti-cancer source & can be used direct or in the combination of presently prescribed drugs for human breast cancer.

6.0 Proposed work plan/Formulations and structure of study:

Year-wise plan of work and targets to be achieved

Year I: Literature survey and course work.

Year II: Selection of natural substances and their preparation for study.

Year III: *In vitro* and *in vivo* experiments of selected and prepared bioactive natural substances with the available different types of breast cancer cell lines and mouse models.

7.0 References:

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